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Research Note

JONATHAN W. WHITE, JR.

Eastern Regional Research Laboratory, Eastern Utilization Research and Development Division Agricultural Research Service, Philadelphia, Pennsylvania 19118

Methyl Anthranilate Content of Citrus Honey

SUMMARY

A photometric method for determination of methyl anthranilate (MA) was adapted for honey. Twenty-one citrus honeys from 3 states and 5 crops averaged 2.87 μg/g (range 0.84–4.37). For 12 non-citrus honeys, apparent MA content averaged 0.07 μg/g (range 0.00–0.28).

INTRODUCTION

Citrus honey is obtained predominantly from the orange, with lesser amounts from grapefruit and lemon. It is collectively handled in commerce as orange honey, originating principally from Florida and California. It is considered a premium honey and possesses a most distinctive and pleasant flavor. To Nelson (1930) its aroma was reminiscent of methyl anthranilate (MA), which is a component oil of orange flowers. He obtained a positive diazotization test for MA in an ether extract of orange honey. Lothrop (1932) later examined 17 honey samples qualitatively by applying the diazotization procedure to an ether extract of the steam distillate from 1 kg honey. Positive tests were obtained for only the three orange honey samples included. Mixtures of orange and tulip-tree honey gave positive tests for MA down to 10% of the former, and were negative at 5%.

More recently, Deshusses and Gabbai (1962) used thin-layer chromatography of a petroleum ether extract of honey to demonstrate the presence of methyl anthranilate in Spanish orange-blossom honey. By comparison of intensity of chromatographic spots, they estimated that 0.4–0.5 ppm of MA were originally present.

Several years ago we adapted a procedure (Anon., 1957) using diazotization and coupling with 1-naphthol-2-sulfonic acid (Sale and Wilson, 1926) for determination of MA in honey. Changes were primarily in reagent concentration and preparation, resulting in a much more stable, sensitive reagent with lower blank absorbance. Since recoveries of added MA in the range of 5–30 ppm were adequate, the procedure was applied to a number of citrus and non-citrus honey samples.

MATERIALS AND METHODS

Honey samples. The 21 samples from the 1956–57 crops were from the collection made for an extensive analytical survey of U. S. honey (White *et al.*, 1962). They were kept in storage at −20°C between receipt and analysis. Other samples were obtained from producers. The cooperation of Wendell Shore and the Superior Honey Company, South Gate, California, is acknowledged for the 1965 samples.

Method for methyl anthranilate in honey. *Reagents: hydrochloric acid, 1N; sodium nitrite,* 1% in water; *hydrazine sulfate,* 3% in water; *1-naphthol-2-sulfonic acid, potassium salt,* dissolve 1.25 g, practical grade, in 50 ml hot water, add a little decolorizing charcoal and filter after a few minutes. Solution is stable in amber bottle at room temperature for at least 2 weeks; *sodium carbonate,* 25 g dissolved in 75 ml water.

Equipment: distillation unit, Parnas-Wagner all-glass micro-Kjeldahl distilling apparatus (Horwitz, 1955) with variable transformer for power input; *photoelectric photometer,* accepting 10-cm cuvettes, 500-mμ filter.

Procedure. Add 5 ml water to 10 g honey weighed to 1 mg, mix, and transfer into the distilling apparatus using 5 ml more water for washing. Distill, reducing voltage from 115 to 85 when foaming begins, and collect 50±1 ml. Condenser water should not exceed 15°C. It is not necessary to submerge the tip of the condenser as described in the original procedure for grape juice. Transfer the distillate to a 100-ml volumetric flask. Use 50 ml water for a blank. To each flask add 2.5 ml HCl and 0.4 ml $NaNO_2$, and let stand 2 min. Add 0.6 ml hydrazine solution, wash down the neck of the flasks with a few ml water, and let stand 1 min. Add 1 ml naphthol sulfonate solution, and immediately follow with 1.5 ml Na_2CO_3 solution. Mix, make to 100 ml, and determine absorbance in a 10-cm cuvette at 500 mμ in the photometer, using the prepared blank for reference. Calibrate against methyl anthranilate solution, 0–50 μg in the 50 ml analyzed.

RESULTS AND DISCUSSION

Effect of sample size. Samples of a citrus honey weighing 5, 10, and 15 g were carried

through the procedure to determine if solids content during the distillation affected the result. As shown in Table 1, the 10-g sample

Table 1. Effect of sample size on methyl anthranilate (MA) value.

Sample wt.	MA (μg/g)
5.012	1.94
5.046	2.04
10.000	2.42
10.000	2.40
15.058	2.34
14.996	2.27

gives maximal yields of MA. All subsequent recovery work was done at this honey concentration.

Recovery of added MA. MA was added to 10-g honey samples in amounts shown in Table 2. These were subjected to the distillation and analysis as described. Recoveries ranged from 86 to 100%, averaging 94.6%. This recovery, in the microgram range, is comparable with that of Sale and Wilson (1926) in recovering MA added to grape juice, in the milligram range.

The analysis of 33 honey samples for MA is shown in Table 3. The analyses were made at different times, those of the 1956–57 crops after 6 years of storage at −20°C. It has been shown elsewhere (White *et al.*, 1961, 1964) that none of the characteristics measured (carbohydrates, acidity, invertase, diastase, color, and HMF content) change during such storage.

Table 3 shows separate averages for the 21 citrus honey samples and the 12 non-citrus honeys. The values are from single determinations. Standard deviation found for duplicate analyses of seven samples was 0.031 ppm. It is quite apparent that methyl anthranilate as measured by this procedure is characteristically present in citrus honeys and absent from non-citrus honey types. The traces shown in other honeys are not necessarily MA. An unexpectedly high value of 1.34 μg/g was obtained for a sample labeled "Chinquapin," from Florida, not shown in the table. This sample was excluded from the table because of the definite possibility that it was in fact mixed with some citrus. Further information on this point, with adequate sample control, is necessary.

Portions of three of the 1957 citrus samples included in Table 3 had also been stored for 6 years at room temperature. Table 4 shows the effect of such storage on MA content. A loss of about 15% of that present per year is evident.

The source of the considerable variation among samples from different areas and seasons is not known. It would not be advisable to set a value for MA content of citrus honey without further investigation of this variability with samples of honey gathered under controlled conditions. At this point, it can be concluded only that citrus honey may contain between 0.84 and 4.4 μg MA per gram honey, averaging 2.87 for 21 samples, and 12 samples of non-citrus honey gave an average of 0.07 μg/g.

Table 2. Recovery of methyl anthranilate added to honey.

Honey type	Methyl anthranilate			
	Added[a] (μg)	Found[b] (μg)	Difference (μg)	Recovery (%)
Citrus	6.95	6.10	0.85	87.8
	12.5	12.35	0.15	98.9
	13.9	12.0	1.9	86.2
	25	24.2	0.8	97.0
Basswood	5.70	5.70	0.0	100
	13.3	12.6	0.7	94.5
	27.8	26.0	1.8	93.5
	38.3	38.1	0.2	99.2
Av.				94.6

[a] Indicated amount added to 10 g honey, then carried through procedure.
[b] Difference between result and amount naturally present.

Table 3. Methyl anthranilate (MA) in honey.

Citrus				Non-citrus			
Crop year	Origin	Type	MA[a]	Crop year	Origin	Type	MA
1956	Calif.	Orange	0.85	1956	Calif.	Vetch	0.07
1957	Fla.	Citrus	3.16	1957	Fla.	Saw Palmetto	0.14
			3.10				
			4.19		La.	Clover	0.00
			3.23		Iowa	"	0.28
			2.77		Col.	"	0.03
			4.21		N.C.	Lespedeza	0.05
			4.01		S.D.	Sweet-clover	0.07
			3.95				
			4.37		Md.	"	0.02
			3.34		Mass.	Cranberry	0.06
1963			1.86		Tenn.	Tulip	0.02
1964			2.34	1958	Fla.	Tupelo	0.05
			2.19	1962	Pa.	Basswood	0.04
			1.42				
1965	Calif.	Orange	3.41		Av. non-citrus		0.07
			3.32				
			3.90				
	Arizona	Orange	0.97				
			0.84				
		Orange-Mesquite	2.86				
	Av. Fla. samples		3.15				
	Av. Calif. samples		2.87				
	Av. Arizona samples		1.56				
	Av. all citrus		2.87				

[a] μg/g, fresh weight.

Table 4. Effect of storage (6 years) on methyl anthranilate content of citrus honey.

	Method of storage			
			Loss	
	Frozen (ppm)	Room temp. (ppm)	ppm	%
1	4.19	1.83	2.36	56.5
2	4.01	1.59	2.42	60.2
3	4.37	1.80	2.57	58.9

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